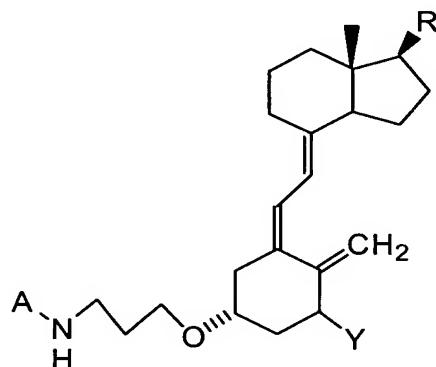


C L A I M S

1. A method of measuring the amount of a 25-hydroxy- or  
5  $1\alpha,25$ -dihydroxy vitamin D metabolite in a sample, comprising measuring binding to or displacement from a vitamin D binding protein of a vitamin D derivative of the formula



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wherein:

R represents a 25-hydroxy side-group of vitamin D<sub>2</sub> or of vitamin D<sub>3</sub>;

15 Y represents hydrogen or hydroxy;

A represents a functional group, coupled via a spacer group, which can be bound by a protein with high affinity;

20 obtained by a method comprising:

a) cyanoethylating the 3-hydroxy group of a vitamin D starting compound in the presence of potassium hydride and tertiary butanol;

b) adding lithium hydride and converting the 25-hydroxy group into the lithium alcoholate and subsequently reducing the nitrile group with lithium aluminium hydride; and

25 c) linking a spacer group together with a functional group A on the amino propylether side chain.

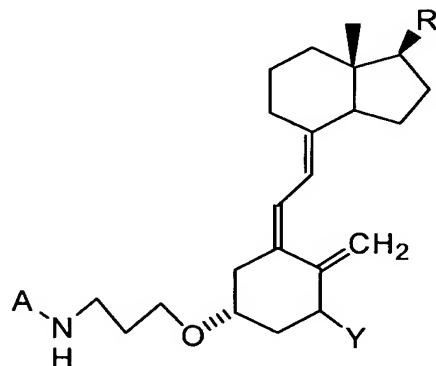
2. The method of claim 1, wherein the method is a competitive immunoassay, selected from the group consisting of radioimmunoassay, enzyme immunoassay, enzyme-linked immunosorbent assay, luminescence immunoassay and fluorescence immunoassay.

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3. The method of claim 1, wherein the method is a sandwich immunoassay, selected from the group consisting of immuno radiometric assay, IEMA/EIA, immuno luminometric assay and immunofluorometric assay.

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15 4. A kit for detection of 25-hydroxy- and  $1\alpha,25$ -dihydroxy vitamin D metabolites comprising a standardized quantity of solid or a standardized solution of a vitamin D derivative of the formula



wherein:

R represents a 25-hydroxy side-group of vitamin D<sub>2</sub> or of vitamin D<sub>3</sub>;

25 Y represents hydrogen or hydroxy;

A represents a functional group, coupled via a spacer group, which can be bound by a protein with high affinity;

wherein the vitamin D derivative is obtained by a

method comprising:

- a) cyanoethylating the 3-hydroxy group of a vitamin D starting compound in the presence of potassium hydride and tertiary butanol;
- 5 b) adding lithium hydride and converting the 25-hydroxy group into the lithium alcoholate and subsequently reducing the nitrile group with lithium aluminium hydride; and
- c) linking a spacer group together with a functional group A on the amino propylether side chain.

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5. The kit of claim 4, wherein the spacing group has the length of 0.9 to 1.5 nm.

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6. The kit of claim 4 wherein A is a biotin group and the spacing group has the length of 0.9 to 1.5 nm.

20 7. The kit of claim 4 comprising a solid phase selected from the group consisting of a microtitration plate, another solid carrier, a microparticle, a polymeric material, and a cellulose.

8. The kit of claim 7, in which the solid phase is a 25 microparticle comprising agarose.

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9. The kit of claim 7, in which the solid phase is a magnetic microparticle.

10. The method of claim 1, wherein displacement of the 30 vitamin D derivative from a vitamin D binding protein is measured and the vitamin D derivative displaces a 25-hydroxy- or  $1\alpha,25$ -dihydroxy vitamin D metabolite from the vitamin D binding protein with a displacement efficiency of approximately 1.